CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

METHYLENEBIS(THIOCYANATE)

Chemical Code # 001185, Tolerance # 50271
SB 950 # 748

JANUARY 15, 1987
Revised 8/10/87, 4/28/89, 8/8/91, 2/3/95, 8/20/97, 10/1/98

I. DATA GAP STATUS

Combined (chronic + onco) rat:	No data gap, no adverse effect
Chronic dog:	No data gap, possible adverse effect
Onco mouse:	No data gap, no adverse effect
Repro rat:	No data gap, no adverse effect
Terato rat:	No data gap, no adverse effect
Terato rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotox:	Not required at this time

Note, Toxicology one-liners are attached

** indicates acceptable study. **Bold face** indicates possible adverse effect.
File name: T981001. Toxicology summary revised by H. Green and M. Silva, 8/8/91; Kishiyama and Gee, 2/3/95; P. Iyer, 8/20/97, 10/1/98.

Note: Previous Summary of Toxicology documents have reflected the grouping of methylenebis(thiocyanate) (# 50271, CC 1185) with methylisothiocyanate (# 50334, CC 392). These are no longer grouped for toxicological testing. This revision reflects those studies on file for methylenebis (thiocyanate).

These pages contain summaries only. Individual worksheets may contain additional effects.

Note: N-948 refers to methylene-bis(thiocyanate).

II. TOXICOLOGY SUMMARY

SUB-CHRONIC, RAT AND MOUSE

042 144257, "NTP Technical Report on Toxicity Studies of Methylene Bis (Thiocyanate) Administered by Gavage to F344/N Rats and in B6C3F1 Mice", (conducted Hazleton Laboratories America, Inc., Rockville, MD.; Leo T. Burka, NTP, Toxicology Report Series, No 32; Dec 1993). Methylene Bis (thiocyanate), approximately 98% purity, administered by gavage in a vehicle of aqueous methyl cellulose to F344/N Rats and B6C3F1 Mice for 2 weeks - 12 dosing days (5/sex) at concentrations of 0, 10, 20, 40, 80 or 160 mg/kg/day or for 13 weeks - 5 days/week (10/sex) at concentrations of 0, 1, 2, 4, 8, or 16 mg/kg/day. Clinical pathology, sperm motility and vaginal cytology were evaluated in the 13 week study. Treatment-related mortality, was observed for the three high dose groups in the 2-week studies. In the 13 week studies, deaths occurred at 2, 4, 8 and 16 mg/kg levels in rats and at the 8 and 16 mg/kg levels in mice. The **stomach** was identified as the **target organ and irritant effects like hyperplasia and hyperkeratosis** were noted. Release of cyanide was also thought to result in acute toxicity at the higher dose levels. For 13-week studies, **NOAEL = 4 mg/kg/day for male rats** and **2 mg/kg/day for female rats and male and female mice** (based on forestomach lesions of hyperplasia and hyperkeratosis). In the genetic toxicity studies, methylene bis(thiocyanate) was not mutagenic in S. typhimurium strains, with or without S9 activation in a preincubation assay, 2 trials. The frequencies of micronucleated normochromatic erythrocytes in the peripheral blood of dosed and control mice were similar. Chemical disposition studies of radioactively labeled methylene bis(thiocyanate) in male F344 rats revealed that more than 90% was eliminated in the urine within 48 hours. In the stomach, the percent remaining in the tissues after a 10 mg/kg labeled dose was increased 10-fold over the amount remaining after 1 mg/kg (0.16 vs. 1.96). This release of cyanide was also thought to result in acute toxicity at the higher dose levels. Blood cyanide concentrations of male rats were increased by at least threefold (296.2 vs. 98 ng/ml) shortly after the 10 mg/kg labeled dose, but were not significantly different from the controls 2 hours after dosing. The individual data were not included so report is not "complete" as submitted. Supplemental data (P. Iyer, 6/18/96).

COMBINED (CHRONIC + ONCOGENICITY), RAT

**044, 051 144267, 149977, "Methylene Bis (Thiocyanate) (MTC) 104 Week Oral by Gavage Carcinogenicity Study in Rats with 52 Week Interim Kill", (C. Atkinson et al, Inveresk Research International, Tranent, EH33 2 NE, Scotland, Report # 7917, 5/14/93), MTC (99.3% pure) was given by oral gavage (7 days/week) for 52 weeks to 20 Sprague-Dawley rats/sex/group or to 50 Sprague-Dawley rats/sex/group at concentrations of 0 (0.5% carboxymethyl cellulose), 0.4, 1.2, and 4.0 mg/kg/day for 104 weeks. Abnormal breathing, lymphocytolysis of the thymus and increased mortality were noted at 4.0 mg/kg dose level. Treatment-related carcinogenic effects were not observed. NOEL = 1.2 mg/kg/day based on increased mortality at 4.0 mg/kg. Previously reviewed as unacceptable, upgradeable upon submission of data from a 13 week dose range finding study (IRI Project No. 436343) and 8 week oral dose range finding study (IRI Project No. 438809). (P. Iyer, 6/18/96). Submission of these studies upgrade the study to acceptable status (P. Iyer, 10/24/96).

051, 149977, "Methylene Bis (Thiocyanate) (MTC) 8 Week Oral Dose Range Finding Study in Rats and Letter report of MTC 13 Week Oral Dose Range Finding Study in Rats", (C. Atkinson et al, Inveresk Research International, Tranent, EH33 2 NE, Scotland, IRI Project Nos. 438089 and 436343. Oct, 1989). See worksheet above.

CHRONIC, DOG

032 111317, 111320 and 111321 (addendum), "Methylene Bis (Thiocyanate) (MTC) 4 Week Oral Dose Range Finding Study in Dogs", (R.J. Greenough and R. Goburdhun, Inveresk Research International, IRI Project No. 637424, 3/29/88). Methylene Bis (thiocyanate), purity not stated, administered by gelatin capsules at concentrations of 0 (gelatin capsule), 5, 10, 20, 40 or 80 mg/kg/day to 1 Beagle dog/sex/group for 4 weeks. Although too few animals per group, treatment related mortality, along with body weight loss and reduced food consumption were observed for the two high dose groups in this range-finding study. Methylene bis (thiocyanate) treatments, at all dose levels irritated the gastrointestinal tract. Individual animal data for the clinical signs noted in study 024 086635 were provided (111321) and demonstrated that an MTD had been reached. Upgraded 086635 to **Acceptable** [Submission of clinical observations (111321) and range finding study (111317)]. Supplemental study (P. Iyer 10/2/95).

****024 086635**, "Methylene Bis (Thiocyanate) (MTC) 52 Week Oral Toxicity Study in Dogs", (Goburdhun, R., Greenough, R.J., Howroyd, P., Inveresk Research International, Musselburgh, EH21 7UB, Scotland, Report # 5892, 5/22/89), Methylene Bis Thiocyanate (99.4% pure) was administered by gelatin capsule for 52 weeks (7 days/week) at analytical concentrations of 0, 0.5, 2.0, and 5.0 mg/kg/day to 4 Beagle dogs/sex/group. Chronic NOEL < 0.5 mg/kg (**Possible adverse effect:** An increase in **hemopoiesis of the sternum/rib** was reported in all treatment levels for females (3 dogs/dose level), associated with a slight (not statistically significant, but dose-related) decrease in erythrocytes, hemoglobin and hematocrit (approximately 10% decrease for all three parameters at the high dose by week 39) at $\geq$ 2.0 mg/kg for males. In females, hemoglobin was decreased in a dose-related manner (not statistically significant), by week 26 (8% decrease at the high dose). In addition, decreased levels of albumin and total protein were reported for 2 and 5 mg/kg animals throughout the dosing period. Clinical signs of emesis (frequency: 20 in controls, 60 0.5 mg/kg, 300 at 2.0 mg/kg and 600 at 5.0 mg/kg) demonstrated that an MTD had been reached. NOT ACCEPTABLE (Request submission of clinical observations and range finding study: IRI Project No. 637424, IRI Report No. 5144), possibly upgradeable. (H. Green & M. Silva, 7/15/91).
Submission of **032 111317, 111320 and 111321** upgrade the study to ACCEPTABLE status (P. Iyer, 10/2/95)

ONCOGENICITY, MOUSE

**043 144266, "Methylene Bis (Thiocyanate) (MTC) 78 Week Oral by Gavage Carcinogenicity Study in Mice" (C. Atkinson et al., Inveresk Research International, Tranent, EH33 2NE, Scotland, Report # 7814, 5/14/93), Methylene Bis (Thiocyanate) (99.3% pure) was administered by oral gavage 78 weeks (7 days/week) at concentrations of 0 (0.5% carboxymethyl cellulose), 0.4, 1.2, and 4.0 (3.0 from week 4) mg/kg/day to 50 CD-1 mice/sex/group. Chronic NOEL = 1.2 mg/kg based on mortality and weight loss at high dose. No evidence for an oncogenic effect. Unacceptable, upgradeable upon submission of data from the 13 week dose range finding study (IRI Project No. 436338) and the 8 week oral dose range finding study (IRI Project No. 438073). (P. Iyer, 6/20/96). Submission of these studies upgrade the study to acceptable (P. Iyer, 8/21/97). Revised on 10/1/98.

052 149986 “Methylene Bis (Thiocyanate) (MTC) 8 Week Oral Dose Range Finding Study in Mice" (C. Atkinson et al., Inveresk Research International, Tranent, EH33 2NE, Scotland, IRI Project Nos. 438073 and 436338, 10/10/89). Test material, methylene Bis (Thiocyanate) (MTC) was milled down to 50-100 microns to aid preparation of homogenous suspensions of low concentration and administered by oral gavage for 8 or 13 weeks (7 days/week) at concentrations of 0 (0.5% carboxymethyl cellulose), 0.1, 0.5, 1.0 and 4.0 mg/kg/day to 10 CD-1 mice/sex/group (for 8 week study) and 0, 1.0, 8, 16 and 24 mg/kg/day to 10 CD-1 mice/sex/group (for 13 week study). Deaths,

adverse clinical signs, reduced body weight gain, reduced food consumption and thickening and erosion of the stomach mucosa were noted in the 13 week study at doses of 8, 16, and 24 mg/kg/day with no notable findings at 1 mg/kg/day. In the 8 week study findings of reduced body weight gain, and mild irritant effects on the stomach were observed at 4 mg/kg/day with no notable findings at 1 mg/kg/day. These effects suggest a local irritant effect on the gastric mucosa with concomitant reduction in food consumption at the doses affected. Premature deaths were seen animals at the 8 mg/kg/day dose and above with marked signs of toxicity in both sexes (deaths, adverse clinical signs, dyspnoea, reduced body weight gain and thickening and erosion of the stomach mucosa). No effects were seen at 1 mg/kg/day. No worksheet. (P. Iyer 8/21/97).

REPRODUCTION, RAT

028 093209, "Methylene Bis (Thiocyanate) (MTC) Two Generation Reproduction Study in Rats", (J.A. Wilson and S.J. Barton, Inveresk Research International, IRI Report No. 7374, 10/30/90). Methylenebis(Thiocyanate) (MTC), purity 99.3%, administered by oral gavage at concentrations of 0, (carboxymethylcellulose), 1.0, 2.5 and 4.0 mg/kg/day. The high dose was 5.0 mg/kg/day for the first 16 days of treatment; but thereafter lowered to 4.0 mg/kg/day due to excessive toxicity. The deaths which occurred in the F0 (4 at high dose) and F1 generation (3 at high dose and 1 at mid dose) were considered treatment related. Additionally 3 F0 animals (1 low dose and 2 mid dose) and 5 F1 animals (2 low dose, 1 mid dose and 2 high dose) died from unknown causes. Probable values for parental and reproductive NOEL = 1.0 mg/kg/day (based on F0 parental deaths and pup survival for F1 generation). UNACCEPTABLE (lack of data). Upgradeable upon submission of data on analysis of the dosing suspension for stability and homogeneity; histopathology results; individual food consumption and body weight (J. Kishiyama and P. Iyer 10/31/95). See 048, 049 146325, 146326 for analytical data, individual body weights and food consumption data, satisfying those deficiencies (Iyer and Gee, 4/23/96).

048, 049 146325, 146326 "Establishment of Methodology for the Analysis of Methylene Bis (Thiocyanate) (MTC) in Aqueous Methyl Cellulose" and "Addendum to Methylene Bis (Thiocyanate) (MTC) Two Generation Reproduction Study in Rats", (S.J. Barton, Inveresk Research International, IRI Report Nos. 338642, 436385, 4/2/96). Methylenebis(Thiocyanate) (MTC), purity 99.3%, administered by oral gavage at concentrations of 0 (carboxymethylcellulose), 1.0, 2.5 and 4.0 mg/kg/day. Deaths in the F0 (4 at high dose) and F1 generation (3 at high dose and 1 at mid dose) appear treatment related, while 3 F0 animals (1 low dose and 2 mid dose) and 5 F1 animals (2 low dose, 1 mid dose and 2 high dose) died from unknown causes. Analysis of the dosing suspension for stability (048 146325) indicates that the actual value of MTC administered is 85% of the nominal. Parental and reproductive NOEL = 1.0 mg/kg/day (nominal) based on F0 parental deaths and pup survival for F1 generation. UNACCEPTABLE. Upgradeable upon submission of necropsy and histopathology results (P. Iyer 4/23/96).

**054 162785 "Histopathology Extension to Inveresk Project #436385 (Methylene Bis (Thiocyanate) (MTC): Two Generation Reproduction Study in Rats), (S.J. Barton and W. Henderson, Inveresk Research International, IRI Report Nos. 16015, 6/18/98). Methylenebis(Thiocyanate) (MTC), purity 99.3%, administered by oral gavage at concentrations of 0 (carboxymethyl-cellulose), 1.0, 2.5 and 4.0 mg/kg/day. Deaths in the F0 (4 at high dose) and F1 generation (3 at high dose and 1 at mid dose) appear treatment related, while 3 F0 animals (1 low dose and 2 mid dose) and 5 F1 animals (2 low dose, 1 mid dose and 2 high dose) died from unknown causes. Histopathology data for F0 animals (terminal and those found dead during study) and F1 animals (only terminal) provided in this report but pup necropsy results previously requested were not submitted. Parental and reproductive NOEL = 1.0 mg/kg/day (nominal) based on F0 parental deaths and pup survival for F1 generation. The compound does not exert significant changes in the reproductive system. ACCEPTABLE. (P. Iyer 9/30/98).

TERATOLOGY, RAT

**035 118202 "Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene Bis (thiocyanate) Administered Orally Via Gavage to Crl:CD*(SD)BR Presumed Pregnant Rats", (A.M. Hoberman, Argus Research Laboratories, Inc., Laboratory Project ID 1419-001, 6/30/89). Methylene Bis(thiocyanate), purity 99.0% administered by gavage (intubation) at concentrations of 0 (aqueous 0.5% [v/v] methylcellulose), 1, 3, or 6 mg/kg to 25 pregnant Crl:CD*(SD)BR pregnant rats/group during days 6 through 15 of gestation. Dosing was inadvertently not given on the final dosing day to 10, 12, 11 and 5 rats in the 0, 1, 3 and 6 mg/kg groups, respectively. Possible adverse effect: dead or resorbed conceptuses averaged 6.4%, 6.5%, 7.8% and 9.9% (no statistical significance) for the respective vehicle control, low, mid and high dose animals that were dosed according to schedule (gestation days 6-15). Dead or resorbed conceptuses in historical controls range from 2.1% to 9.4%. Also, the same high dose animals exhibited abnormal respiration sounds (rales); and lower body weight (-3%), body weight change (-15%) and food consumption (-5%, [g/kg/day]). Maternal and Developmental NOEL = 3 mg/kg/day. ACCEPTABLE. (J. Kishiyama and P. Iyer, 1/12/96).

TERATOLOGY, RABBIT

037 118206, "Developmental Toxicity (Embryo/Fetal Toxicity and Teratogenic Potential) Study of Methylene Bis (thiocyanate) Administered Orally (Stomach Tube) to New Zealand White Rabbits", (A.M. Hoberman, Argus Research Laboratories, Inc., Project I.D. 1419-002, 6/30/89). Methylene bis (thiocyanate), purity 99.0% administered by gavage (intubation) at concentrations of 0, 1, 3.5 or 7 mg/kg (5 mg/kg/day) to 20 artificially inseminated New Zealand White rabbits/group during days 6 through 18 of gestation. After 2-5 doses, due to mortality, the 7 mg/kg dose level, was reduced to 5 mg/kg. Body weight gain and food consumption was lower and adverse clinical signs (decreased motor activity, impaired and or loss of righting reflex, ataxia, tremors, bradypnea, dyspnea, dried feces and localized alopecia) were observed for the high dose group; **Maternal NOEL = 3.0 mg/kg/day (actual) based on lowered body weight gain and adverse clinical signs. **Developmental NOEL** > 5 mg/kg/day (nominal). Dose levels were adjusted according to the results of the dosing solution analysis. ACCEPTABLE. (J. Kishiyama and P. Iyer, 10/24/95).

MUTATION, GENE
Microbial Systems

**036 118204, "Salmonella/Mammalian-Microsome Plate incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay", (T.E. Lawlor and V.O. Wagner, Microbiological Associates, Inc., Study number T8013.501014, 8/12/88). Methylene Bis (thiocyanate), purity not stated, tested in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 with and without Aroclor-induced rat liver activation at 0 (DMSO), 0.1, 0.3, 1.0, 3.3, 10 and 20 ug/plate and at 0 (DMSO), 0.3, 1.0, 3.3, 10, 33 and 50 ug/plate, respectively. Toxicity was slight to moderate at 10.0 ug/plate and extreme at 20 and 50 ug/plate. There were no evidence for increase in reversion rate for either the initial or confirmatory assay. Acceptable (J. Kishiyama and P. Iyer, 11/16/95).

014 043569 "Mutagenicity Evaluation of (N-948) Sample #200: Final Report." (10/26/1976, Litton Bionetics, Project No. 2683, T-6082.) N-948, no purity stated, gold granules; tested with Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 with and without Aroclor-induced rat liver activation, also Saccharomyces cerevisiae D4; 0, 0.1, 1.0, 10 or 100 ug/plate with and without activation, single plate, single trial; toxic at 100.0 ug/plate; no evidence for increase in reversion; unacceptable (no repeat trial, single plate, no purity of test article.) Gee, 7/10/86.

014 043574 "Mutagenicity Evaluation of N-948" (VR 6/28/77, Ardsley Composite): Final Report. (10/1977, Litton Bionetics, Project No. 20838, T-6299) N-948, tan crystals, no purity stated; tested in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 with and without

Aroclor-induced rat liver activation at 0, 0.01, 0.1, 0.5, 1.0, 10.0 and 100.0 ug/plate, one plate, one trial; toxicity at 10.0 ug/plate; no evidence for increase in reversion rate; unacceptable (no purity of test article, single trial, single plate.) Gee, 7/10/86.

** 014 043579 "Mutagenicity Evaluation in Salmonella typhimurium." (8/26/1980, Stauffer Chemical Company, Report No. T-10043) N-948, EHC-0008-6-6, no purity stated, yellow crystals; Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100; tested with and without Aroclor-induced rat or mouse liver activation and phenobarbital-induced rat or mouse liver activation; triplicate plates, multiple trials; without activation, 0, 0.04, 0.13, 0.40, 1.20, 3.6, 3.7, 11.11, 33.33, 100.0 and 300.0 mg/plate - toxic > 3.7 mg/plate; with Aroclor-induced rat liver, 3 trials, 0, 0.14, 0.41, 1.23, 3.7, 11.11, 33.33, 100 and 300 ug/plate - toxic > 33.33 mg/plate; phenobarbital-induced rat liver, 1 trial, 0, 0.12, 0.37, 1.11, 3.33 and 10.0 ug/plate - slight toxicity at 10.0 ug/plate; Aroclor-induced mouse liver, 1 trial, 0, 0.12, 0.37, 1.11, 1.23, 3.33, 3.7, 10.0, 11.11, 33.33 and 100.0 ug/plate - toxic at 100.0; phenobarbital-induced mouse liver, 1 trial, 0, 0.12, 0.37, 1.11, 3.33 and 10.0 ug/plate - no cytotoxicity; acceptable. No evidence for increase in reversion rate. Gee, 7/11/86.

017 051084 Summary tables only - see 043579.

Other Systems

014 043578 "Mutagenicity Evaluation of N-948, Lot #685 in the Sex-Linked Recessive Lethal Test in Drosophila melanogaster: Final Report." (7/1979, Litton Bionetics, T-10014) N-948, no purity stated, yellow crystalline powder; fed in DMSO-1% sucrose to wild type males - number not clear but general protocol states minimum of 200 at 0.025 or 0.05 mg/ml stated to be 1/4 and 1/2 the LD50 - no data included; mated to Basc females, 1:3, four broods of 2-3-3-4 days; F_2 examined for wild type males; no evidence for adverse effect is reported; unacceptable (no purity of test article, doses stated to be based on LD50 but no data, number of males not clear, use of DMSO is discouraged, concurrent control failed due to sterility of males treated with EMS.) Gee, 7/10/86.

014 043575 "Mutagenicity Evaluation of N-948 (Batch # 435) in the Mouse Lymphoma Forward Mutation Assay: Final Report." (10/1977, Litton Bionetics, Project No. 20989, T-6411) N-948, purity not stated, technical grade, yellow crystals; tested with and without Aroclor-induced rat liver activation, 4 hours incubation, at 0, 0.05, 0.1, 0.2, 0.39 or 0.78 without activation and 0, 0.78, 1.56, 3.13, 6.26 or 9.37 ug/ml with activation; cytotoxicity at 0.78 without and at 6.25 with activation; unacceptable (single trial, no purity stated).

Nonactivation	Control	0.05	0.1	0.2	0.39	0.78 ug/ml
Mutation Frequency	13.2, 12.3	12.9	23.5	33.3	32.7	39.3
% Relative Growth	100, 57.3	37.1	56.0	26.5	22.1	5.0

Activation	Control	0.78	1.56	3.13	6.25	9.37 ug/ml
Mutation Frequency	24.6, 7.0	38.6,	36.3	57.0	62.8	-
% Relative Growth	100, 123.6	93.5	111.3	114.7	59.7	8.4

Weak positive effect + activation. Gee, 7/10/86.

**** 014 043580** "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test." (10/20/1980, Stauffer Chemical Company, Report No. T-10140.) N-948, EHC-0008-6-6 and EHC-0008-53 (lot no. 112, 96-97%); tested with and without Aroclor-induced rat liver activation; four trials, 4-hour incubation with test compound at 0, 0.0125, 0.025, 0.5, 0.1 and 0.2, no activation, and

0, 0.125, 0.25, 0.5, 1.0 and 1.0 ug/ml, trial 1 with slight cytotoxicity at 0.2 ug/ml, -S9; concentrations in trial 2 were 0.00625, 0.0125, 0.025, 0.05 and 0.1, -S9, and 0, 0.25, 0.5, 1.0, 2.0 and 4.0 ug/ml with slight cytotoxicity at 4.0, +S9; in trial 3, 0.0313, 0.0625, 0.125, 0.25 and 0.5 ug/ml, -S9, and 0, 0.625, 1.25, 2.5, 5.0 and 10.0 ug/ml +S9; in trial 4, 0.3, 0.4, 0.5, 0.6 and 0.7, no activation, and 0, 4.0, 6.0, 8.0, 10.0 and 12.0 ug/ml, with activation; marginal increase in mutation seen with activation in trials 3 and 4 - especially trial 4.

Trial 3	Solvent	Medium	0.625	1.25	2.50	5.0	10.0	ug/ml
Mut. Frequ.	39, 31	49, 51	53	58	54	50	74	
% Rel. Gro.	100, 100	82, 102	84	66	72	70	18	

Trial 4	Solvent	Medium	4.0	6.0	8.0	10.0	12.0	ug/ml
Mut. Freq.	16, 14	19, 22	29	43	37	39	44	
% Rel. Gro.	100, 100	95, 123	81	63	69	31	19	

Weakly positive for mutagenicity with activation. Gee, 7/11/86.

017 051082 Purity data on N-948 from Stauffer for multiple studies. The data were requested for completing reports such as 43579 and 43580 which were upgraded to acceptable.

SUMMARY: Data indicate that thiocyanates are negative for mutagenicity in bacteria and yeast but weakly positive in mouse lymphoma (two reports) but negative in Chinese hamster V79/HGPRT assay. Gee, 8/87.

MUTATION, CHROMOSOME

** 014 043581 "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test." (10/20/1980, Stauffer Chemical Company, Report No. T-10140) N-948, EHC-0008-6-6 and EHC-008-53 (lot no. 112, 96-97%); tested for chromosomal aberrations at 0, 0.0032, 0.0063, 0.0125, 0.025 or 0.05 ug/ml without activation, 4 hours, and at 0, 0.25, 0.5, 1.0, 2.0 and 4.0 ug/ml with rat liver activation, 4 hours, in trial one and in repeat at 0, 0.032, 0.063, 0.125, 0.25 and 0.5 ug/ml without activation and 0, 0.312, 0.625, 1.25, 2.5 and 5.0 ug/ml with activation, 4 hours incubation each with harvest 24 hours postexposure; no evidence of increase in aberrations in 50 cells scored per concentration; acceptable Use of a single time of harvesting is not the most desirable and the scoring of only 50 cells (in some samples, fewer) does not conform with the current Gene-Tox guidelines. Gee, 7/11/86.

017 051083 One-page of cytotoxicity data for #'s 043581 and 043582.

017 051082 Purity and characterization data for lot 112 for # 43581.

014 043577 "Mutagenicity Evaluation of N-948, Lot #685 in the Rat Bone Marrow Cytogenetic Assay: Final Report." (7/1979, Litton Bionetics, Project No. 21092) N-948, no purity stated, yellow to light orange powder; given by oral gavage at 0, 1, 3.3 or 10 mg/kg in a single dose or in 5 consecutive daily dosings; to 8 males per group per sacrifice time; sacrificed at 6, 24 or 48 hours after acute dosing and 6 hours after final dosing in subchronic schedule; triethylenemelamine as positive control; up to 50 cells scored per slide; increased number of cells with aberrations and increased frequency at 10 mg/kg, 24 and 48 hour sacrifices; unacceptable (use of males only is not justified, no evidence for MTD or for marrow cytotoxicity for dose selection with no mitotic index information.) Gee, 7/11/86.

014 043584 "Mutagenicity Evaluation in Bone Marrow Cytogenetic Analysis in Rats - N-948." (10/30/1981, Stauffer Chemical Company) N-948, lot # 112, purity at 97%; given by oral gavage

to 8 males per group per sacrifice time at 0, 3.3, 10 or 30 mg/kg in a single dosing or in 5 consecutive daily doses (subchronic); sacrifice at 6, 24 or 48 hours after acute dosing and five days after initial dosing in the subchronic; triethylenemelamine as positive control; unacceptable (use of a single sex, no individual data, no evidence of MTD or cytotoxicity in bone marrow as justification for dose levels.) Study was conducted to confirm results in #43577 above with inclusion of a higher dose but the effect at 10 mg/kg was not repeated at 30 mg/kg, making the significance of the finding in 43577 questionable. Gee, 7/11/86.

**** 014 043582** "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test." (10/20/1980, Stauffer Chemical Company, Report No. T-10140) N-948, lot #112, 96-97%, yellow crystals; tested at 0, 0.0032, 0.0063, 0.0125, 0.025 and 0.05 ug/ml without activation for sister chromatid exchange and at 0, 0.5, 2.0 and 4.0 with rat liver activation in first trial; concentrations were increased in the second trial at 0.032, 0.125 and 0.25 ug/ml without activation and to 0.312, 0.625, 1.25, 2.5 and 5.0 ug/ml with activation; a statistically significant increase in sister chromatid exchanges per cell occurred without activation but not with activation; 20 or fewer cells scored per concentration; acceptable; explanation why so few cells (20 or fewer) were evaluated for a given concentration is explained in the rebuttal - statistics would be much better if >50 cells were counted. Gee, 7/11/86.

017 051083 One-page report of cytotoxicity data for #'s 043581 and 043582.

017 051082 Purity and characterization of Lot 112 for # 043582.

**036 118203, " Micronucleus Cytogenetic Assay in Mice", (D.L. Putman, Microbiological Associates, Inc., Study number T8013.122, 10/18/88). Methylene Bis (thiocyanate), purity not stated, was administered by a single IP injection at concentrations of 0 (1% CMC), 0.3, 1.3 or 2.6 mg/kg to 5 ICR mice/sex/group/sacrifice time. Sacrifice time was scheduled at 24, 48 or 72 hours after treatment. The incidence of micronucleated polychromatic erythrocytes in bone marrow was not increased with methylene bis (thiocyanate) treatments. Acceptable (Kishiyama, J. and Iyer, P., 11/10/95).

SUMMARY: A positive cytogenetic effect <u>in vitro</u> in male rats at 10 mg/kg was not confirmed at 30 mg/kg, albeit at a different facility. Neither study was considered acceptable. Methylenebis(thiocyanate) was negative in mouse lymphoma cells in a study conducted concurrently with the gene mutation study, which was positive. The same laboratory, however, reported an increase in sister chromatid exchanges in mouse lymphoma, indicating a positive effect for chromosome damage. Gee, 8/87. The in vivo study 036 118203 was acceptable and did not demonstrate an increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow, indicating a negative effect for chromosomal damage with methylene bis (thiocyanate) treatments. P. Iyer 1/17/96.

MUTATION, DNA

**036 118205, "Unscheduled DNA Synthesis in Rat Primary Hepatocytes", (R.D. Curren, Microbiological Associates, Inc., Study number T8013.380, 8/12/88). Methylene Bis (thiocyanate), purity not stated, tested at concentrations of 0 (DMSO), 0.03, 0.10, 0.3, 1.0, 1.5, 3.0, 6.0 or 10.0 µg/ml with Fischer 344 adult male rat primary hepatocytes. Exposure time was 18-20 hours. LDH release data indicate that toxicity was not apparent at doses 0.3 mg/ml and lower. Methylene Bis (Thiocyanate) at doses tested did not significantly increase the average net nuclear grain counts. Acceptable (J. Kishiyama and P. Iyer 11/15/95).

014 043576 "Mutagenicity Evaluation of N-948 (Batch # 435) in the In Vitro Transformation of

Balb/3T3 Cells Assay: Final Report." (5/1978, Litton Bionetics, T-6413) N-948, no purity stated, batch # 435, yellow crystals; without activation only; tested at 0, 0.078, 0.156, 0.312, 0.625 and 1.25 ug/ml, incubated 72 hours; 10 plates for foci scoring per concentration; concentration selection stated to be based on preliminary cytotoxicity tests but no data is presented; unacceptable but possibly upgradeable (no purity of test article, no evidence of cytotoxicity or justification of concentrations used - in Record # 43583, 0.2 ug/ml was toxic, no activation system included.) No evidence for transformation induced by N-948. Gee, 7/10/86.

** 014 043583 "Morphological Transformation of Balb/3T3 cells." (1980, Stauffer Chemical Company, Report No. T-10141) N-948, EHC-0008-6-6, "assume 100%", yellow crystals; tested for transforming ability without activation at 0, 0.05, 0.06, 0.07, 0.08 and 0.10 ug/ml, exposed for 3 days; no evidence for increase in foci is reported; acceptable. Gee, 7/11/86.

NEUROTOXICITY, HENS

No studies currently on file. Not required at this time.